Palm Readings

Scientists at the University of Washington in Seattle are taking technology that has been in common laboratory usage for over 15 years and adapting it in ways that may literally take the laboratory into the field in the palm of a researcher's hand. Once the size of an old-fashioned console television, the technology known as surface plasmon resonance (SPR) has now been integrated into a sensing unit that is no bigger than a conductor's baton. Such units may be used for environmental monitoring, agricultural pesticide and antibiotic monitoring, food additive testing, military and civilian airborne biological and chemical agent testing, and real-time chemical and biological production process monitoring. SPR may also be useful in performing medical diagnoses—because there is generally no sample preparation involved, the time from sample application to result is frequently as short as 2–5 minutes, which is of particular value in a critical medical setting.

"Surface plasmon resonance sensors have been used for about 15 years in research labs," explains Sinclair Yee, head of the team at the University of Washington's College of Engineering that developed the new technology, "but the technology was just too cumbersome and too complicated, not to mention too expensive, to bring it out of the lab. The potential benefits of these sensors to medicine, to environmental monitoring, and manufacturing is tremendous, so we set out to develop an instrument that is both portable and inexpensive."

Surface Plasmon Resonance

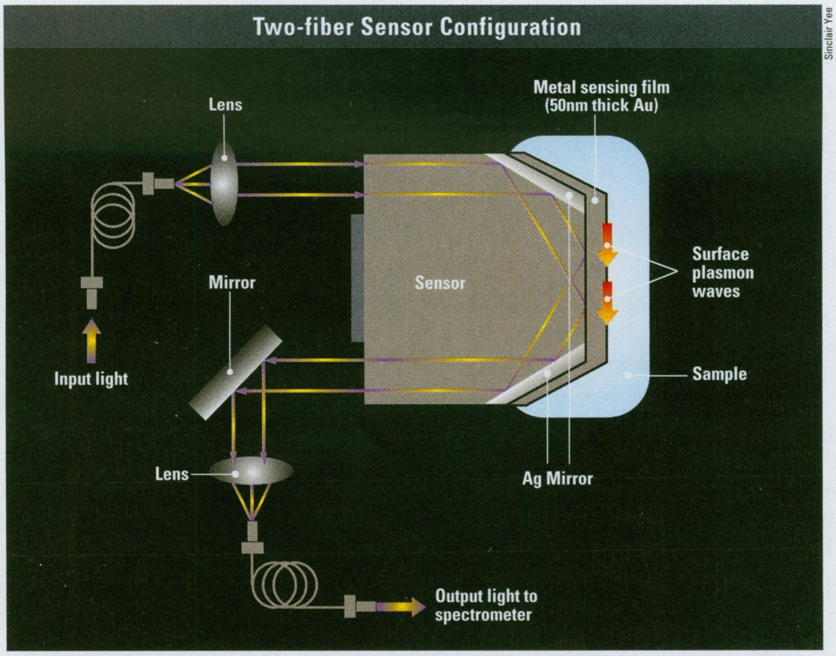
SPR relies on the physical principle that the energy carried by photons of light can be transferred to electrons on a metal surface. The wavelength at which this energy transfer takes place is characteristic of both the metal and the environment of the metal surface, which is illuminated. When there is a match, or "resonance," between the energy of the light photons and the electrons of the metal surface, an energy transfer takes place, and that transfer can be observed by measuring the amount of light reflected from the metal surface. All of the light at most wavelengths is reflected except the resonant wavelength, where almost all light is absorbed, and that absorption is measured by a reflectance spectrophotometer.

When the light transfers its energy, a "plasmon," a group of excited electrons that behaves as a single electrical entity, is created. This plasmon generates an electrical field extending a minute distance above and below the metal surface. What makes SPR such a valuable real-time analytical tool is that any change in the chemical composition of the environment within range of the plasmon field causes a change in the wavelength of light that resonates with the plasmon. Thus, by measuring such changes, researchers can detect the presence of chemicals in the environment.

"In fact," Yee says, "this technology is so sensitive, we've been able to detect changes in optical properties of the substances being tested on the order of 10^{-6} refraction index units, and some heavy metals we've detected as low as 10 parts per billion."

As developed by Yee and his team, the sensor unit consists of a single probe, coupled to a separate unit that performs the analysis. The probe contains a glass fiber core less than 0.5 mm in diameter. When the unit is activated, white light is transmitted down the fiber core to the tip of the probe, where the sensor surface is in contact with the sample being tested, and light is absorbed by the sample. The wavelength at which most of the light is absorbed into the sample—rather than reflected back to the probe—is called the wavelength of resonance and depends on the composition of the sample. Computer analysis of the reflected light can determine the presence and concentration of specific chemicals.

Unlike traditional SPR systems, which consist of a prism with a thin layer of highly conductive metal such as gold deposited onto one face to form a sensing surface, the new sensor probe is designed around a number of facets polished onto a glass rod, making this probe simpler and less expensive than traditional probes and reducing the construction difficulties associated with them.



In the SPR sensor. Light is emitted from an output leg and collimated by an achromatic lens. It then reflects off the probe surfaces onto the sensing surface and triggers a surface plasmon wave. This results in a reflection spectrum that is reflected off the surfaces on the opposite side of the sensor, back into the optical fiber, and into a computer for data analysis.

Sinclair Yee

The sensor developed by Yee and colleagues works by focusing white light into a fiber-optic coupler. The light is emitted from one of the output legs and collimated by an achromatic lens. The light reflects off the tapered probe surfaces and hits the sensing surface, which has been exposed to a sample. A surface plasmon wave is triggered, resulting in a reflection spectrum that is then reflected off the surfaces on the opposite side of the sensor, back into the optical fiber, and then into a computer for data analysis.

"What we realized at the onset of our research was the [potential for] miniaturization of the sensor system," Yee says. "The lab versions were not only bulky, they were tremendously expensive, which limited their use to some select research laboratories. Additionally, a handheld probe allows the sensor technology to be placed into a fluid sample, rather than having to transport the sample to a large laboratory machine. Our main step was to replace what was a relatively complex optical system with something smaller and less expensive. After five years of research, we were able to develop an SPR sensor on an optical fiber surface, which eliminated a lot of optical components. The fewer components a system has, the easier it is to use, the less elevated a training level is required to operate it, and the fewer alignment problems you're apt to encounter."

Optical fiber was an answer, Yee says, but it was not the whole solution. "You get into some rather arcane manufacturability issues," he admits. One such issue, according to Yee, was that this SPR requires cylindrical geometry, but the technology for making it in a batch is a planar technology. Says Yee, "The first thing we did was to develop an SPR sensor on a microscope slide, and then we took that approach to making the probe, which allowed us to overcome these manufacturability issues while still retaining flexibility and cost reduction."

Collaborative Technologies

Combining the SPR with one or more current chemical analysis technologies can help to assure the detection of target chemicals. One such technology is known as molecular imprinting. Molecular imprinting, says Christophe Sevrain, president of the Portland, Oregon, biotechnology company Ikonos Corporation, which has licensed the new sensor technology, involves the creation of what he calls a "plastic antibody."

In this technology, Sevrain says, a thin film polymer is created with "locks" that are actually binding sites for specific chemicals—only the molecule of a specific chemical will bind to a particular site. These molecules act as templates during polymerization, after which they're chemically removed, leaving sites that are the equivalent of an "induced molecular memory" that can selectively recognize the imprint species. Such plastic antibodies can be attached to the surface of the sensor to enhance recognition of specific chemicals.

"You can do this kind of thing with a live antibody," says Sevrain. "It's done all the time. But the plastic antibody is very low cost, it has an almost infinite shelf life, and it's immune to the problems caused by heat, cold, shock, vibration, and so on that would affect a living antibody. This is an inert substance, it can be sterilized, and depending on whether the binding is covalent or [not], the sensor can be cleaned *in situ* with water, heat, or even a low voltage electric current. None of this applies to a live antibody."

In a paper by Klaus Mosbach and colleagues of the department of pure and applied biochemistry at Lund University in Sweden that was published in the June 1997 issue of *Analytical Chemistry News & Features*, the authors state, "One of the advantages of molecular imprinting is that imprints can be made of compounds against which it is difficult, if not impossible, to raise antibodies. The use of animals—often necessary with antibody production—is avoided, and the scale-up for bulk manufacture is easily done. Thus, benefits are reaped from practical, ethical, and economical points of view."

"It's a way to enhance the effectiveness of the sensor," Yee says, "as well as allow us to select the species [of chemical] to be detected. Molecular imprinting enables the sensor to be phenomenally selective for given molecules." Molecular imprinted polymers have been prepared with affinities for such things as proteins, amino acid derivatives, sugars and their derivatives, vitamins, pesticides, and pharmaceuticals including theophylline, morphine, diazepam, and pentamidine.

The polymer can be reused, according to Sevrain, depending upon the type of bonding that takes place. "If it's covalent bonding," he explains, "[such as] what you'd want to have if you were dealing with a toxin and you wanted to make sure the molecule stayed in place so that you could later dispose of the sensor, then we're talking single use. On the other hand, if you're in a somewhat nasty industrial situation, where you can't get to the sensor to replace it easily, you'd like to get hundreds, maybe thousands, of uses. That's when a weaker ionic bond might be appropriate."

To date, Sevrain says, the easiest molecules to imprint have been on the order of a molecular weight of 20–400, which encompasses most drugs of abuse, he adds, as well as most therapeutic and specialty drugs.

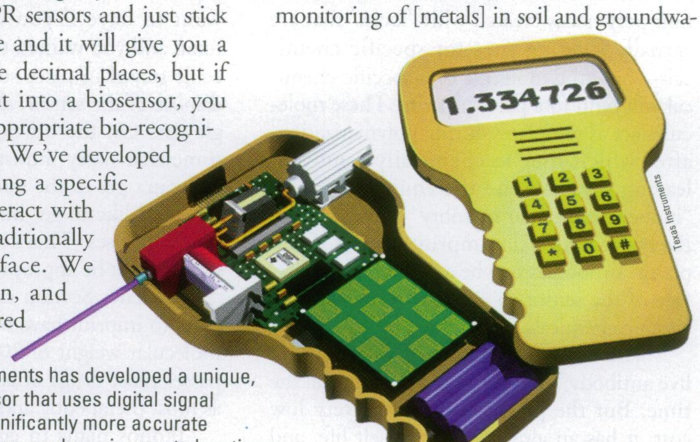
Ikonos plans to commercially produce sensors combining Yee's technology with molecular imprinting technology by late 1998. The company is currently designing and building prototype sensors, tailored to individual client needs, at a cost of $50,000–100,000 per sensor. Clients currently range from petrochemical processors and drug manufacturers to police departments and veterinary clinics. The sensors, says Sevrain, can accurately detect some molecules in the range of tens of parts per billion concentration.

These sensors, Sevrain says, may be most important in medical settings where real-time analysis is vital. For example, he says, if someone has had a heart attack and minutes are important, "you don't want to have to wait for reports to come back from a lab. This type of sensor will give you real-time figures on such things as the enzymes released by the breakdown of heart muscle tissue during a heart attack. Those few moments could save many lives."

According to Sevrain, there are few practical limitations to the number of different test sites that can be incorporated into a single sensor. "Give us a surface 100 microns by 100 microns," he says, "and that's plenty of room [for each site]. However, with a surface of say, 1 cm by 1 cm, you could, in theory, have 100 different test subjects." The problem, he continues, is having the technology to analyze the results of all those tests in a timely enough manner to make the sensor practical for on-site testing of large numbers of substances.

In another collaborative technology, Clement Furlong, a University of Washington professor of molecular genetics, is working to develop various chemical binding agents for use in SPR systems. "By putting a chemical layer on top of the metal," Yee explains, "that layer becomes sensitive to whatever chemical is in the solution you're testing. It's a way to let us look at the concentration, and the dynamics of the binding process."

For example, Furlong says, "You can take one of these SPR sensors and just stick it in a cup of coffee and it will give you a sugar reading to five decimal places, but if you want to make it into a biosensor, you need to attach the appropriate bio-recognition molecules to it. We've developed a procedure involving a specific protein that will interact with gold, which is the traditionally preferred SPR surface. We modify that protein, and then attach the desired antibodies to that surface to create a sensing surface that is not only robust, but also stable, sensitive, and has a high degree of specificity for whatever substance we're testing for. And antibodies can be raised for any number of organic substances."



Tiny tool. Texas Instruments has developed a unique, fully integrated biosensor that uses digital signal processors to make significantly more accurate calculations and real-time analyses in remote locations.

Combined with SPR, Furlong's system is sensitive enough to detect metals such as lead, arsenic, and copper in concentrations as low as 10 parts per billion, making it ideal for monitoring soil and water pollution, or for real-time monitoring of chemicals and microorganisms in marine environments. Furlong says these sensors also would be ideal in the field diagnosis of heart attacks. "Paramedics and emergency room doctors need to know right away if a patient is having a heart attack," Furlong explains. "Fifteen years ago, cardiac enzyme blood tests took as long as eight hours, and even today, they still have to go to the lab and back. Our sensors could be in every ER, every ambulance, to provide instant, on-site bloodwork."

Yee says, "I think the commercial applications of this technology are tremendous. I can see it being used, for example, in manufacturing beer or wine, to track the fermentation process. It could also be used to detect biowastes, in the food industry to detect *Salmonella* contamination, for environmental monitoring of [metals] in soil and groundwater, in the petrochemical processing industry . . . and, of course, in the medical arena."

Jose Melendez, manager of analytical sensor activities in the Components and Materials Research Center at Texas Instruments in Dallas, Texas, which is using SPR technology to develop its own miniaturized sensors, cautions that there are many approaches to using this technology. "Our sensor can use either molecular imprinting, chemical antibodies, or any number of other possibilities, and it doesn't rely on fiber optics. Instead, we have a fully integrated [system in which] there's no need to worry about the alignment of outside sources such as light." The Texas Instruments sensor uses digital signal processors, the engines behind the digitization of electronics end equipment, to perform analysis that normally would require a laptop computer or laboratory equipment.

"I think our system has another advantage," says Melendez, "in that not only has it been designed with fabrication techniques consistent with high-volume opto-electronic manufacturing, the system architecture is also such that there are no optical connections to be made, only electrical. You just plug our sensor into a circuit board." The Texas Instruments sensor is not yet in commercial production, but the company expects to exhibit a fully self-contained handheld biosensor system at an analytical measurement show in March 1998.

SPR technology, Sevrain says, "is, quite simply, a revolution in sensor instrumentation much like the revolution in computer technology. Just as computers that were once huge, tremendously expensive, and accessible only to a few have become easily portable and commonplace, [this] sensing technology enables us to put sophisticated chemical analysis capabilities in the hands of many more people."

Lance Frazer

Suggested Reading

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